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Survival of bioaerosols in HVAC system photocatalytic filters

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ABSTRACT

The survival of an experimental bioaerosol in photocatalytic and non-photocatalytic filters was investigated under UV-A or UV-C radiation exposure. To ensure the generation of reproducible bioaerosols in real operating conditions of Heating, Ventilation and Air Conditioning (HVAC) systems and their homogeneous coating on filters, a reactor with an optimal geometry was developed. The bacterial aerosol was generated from standardised bacterial suspensions and the Gram-negative bacterial model *Escherichia coli* was used as the experimental organism. The bioaerosol was characterised in terms of particle size distribution, reproducibility and stability over time.

The coating of aerosolised cells on the filters was then studied. The total number of aerosolised *E. coli* cells was different from the amount of cultivable bacterial cells extracted from filters, probably resulting from the stress of the aerosolisation process. Damages to the outer membrane of *E. coli* cells were clearly observed through SEM and epifluorescent microscopy analysis. The influence of important physical parameters in the reactor-aerosolisation duration, frontal velocity and relative humidity- on the amount of aerosolised cells coated on filters were also investigated.

Finally, the effects of UV-A or UV-C radiation were assessed on aerosolised microorganisms coated on HEPA photocatalytic filters and on photocatalytic or non-photocatalytic filters containing activated charcoal. The HEPA photocatalytic filters demonstrated a better disinfection efficiency with full damages of the aerosolised bacterial cells, probably resulting from an optimal contact between TiO_2 coating and the microorganisms. In contrast, the use of filters with activated charcoal resulted in the apparition of an inactivation threshold that could be attributed to the penetration of aerosolised cells within the activated charcoal layer and the absence of contact with the photocatalyst. However, UV-C photocatalysis was able to inactivate faster and, at the same time, mineralise biological pollutants than UV-A.

1. Introduction

Indoor air quality (IAQ) has become a major public health concern and it potentially poses a threat to the health of all types of populations. Unlike outdoor air pollution, extensively studied and upon which a great deal of research has already been published, indoor air pollution and its effects, albeit stronger in some cases, are often underestimated and poorly controlled. However, these effects can potentially be managed and addressed efficiently [1].

Since the 1970s, the need for greater energy efficiencies as a consequence of worldwide and repeated energy crises has led to the design of increasingly airtight building envelopes, often at the expense of air circulation and therefore indoor air quality. This trend has in turn rendered the exchange and proliferation of airborne microorganisms much easier. These microorganisms have evolved jointly not only with humans but also with their habitat. Furthermore, indoor environments are generally harmless to microorganisms: host availability (humans), presence of humidity and dust acting as nutrients, and an efficient vector of proliferation (air) are some of the factors that can promote and maintain microbial colonisation of indoor air. Bioaerosols are defined as biological airborne particles including microorganisms (bacteria, fungi, viruses, pollens), their fragments and the by-products of their

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metabolism, agglomerated either with each other or with non-biological particles [2,3]. Particle aerodynamic diameters can range from 0.02 to 30 μm. It has now been proven that lengthy or intense exposures to these bioaerosols in confined environments can lead to various health hazards and affect usual human activities, thus making indoor air treatment critical [4,5].

Biological treatment of indoor air is an emerging research theme. Indeed, the removal of biological agents remains a complex issue: the main idea is the treatment of a mix of different types of microorganisms, whether pathogenic or not, often at low concentrations, and for which no regulatory exposure threshold exists. None of the traditional technologies for indoor air decontamination, such as activated charcoal filters, HEPA (High Efficiency Particulate Air) filters with or without UV radiation, ozonation, air ionisation, are completely effective [6]. Moreover, these technologies still need further investigation, especially in terms of generation of toxic by-products and potential health hazards resulting from such exposure.

Ventilation of buildings is usually handled by air treatment units, namely HVAC systems through the extraction of fouled air and the inflow of fresh air previously filtered and thermally treated. These systems allow the reduction of pollutant inflow from outside as well as the dilution of the pollutants already present in the indoor air. Filtration is an economical and efficient method for improving IAQ and is therefore commonly used. However, the filters used in HVAC systems tend to accumulate over their lifetime organic matter, dust as well as aerosolised microorganisms [7,8]. Therefore, it is essential to ensure that these filters do not themselves become a source of microorganism colonisation under specific conditions such as high humidity, poor design or maintenance or accumulation of organic compounds as source of nutrients [9]. This ability of microorganisms to survive in filtration materials, albeit a high source of degradation and of allergens, toxins and COV generation, still remains poorly addressed [10]. Thus, it would contribute to the role of ventilation systems in airborne transmission of infectious agents [11,12].

Like for every other major air treatment technology under development, the efficient design, dimensioning and operation of HVAC system is a major issue. The aim is not only to trap or inactivate the microorganisms present in the indoor air, but also to alter them irreversibly, as well as their by-products arising from their degradation. In this context, the combination, within air treatment units, of both the photocatalytic and the filtering process is a promising technology that would combine the benefits of both processes to efficiently trap and alter chemical and microbiological pollutants through total mineralisation. Photocatalysis is an advanced oxidation process, based on a photocatalyst that, once activated under UV radiation, alters organic matter through the formation of reactive species. Its efficacy for microorganism inactivation has drawn a great deal of attention in both the academic and industrial fields for the past 25 years [13–15]. Titanium dioxide photocatalysis has been widely applied for the disinfection of aqueous liquid phase and more recently, the removal of biological airborne pollutants in photocatalytic reactor has been reported [16–21]. A number of companies also market photocatalytic devices for air disinfection in order to control contamination in various indoor environments. However, few studies have taken an interest in the survival of aerosolised microorganisms in air filters [9,22].

The main contribution of this research is to provide a better understanding of the parameters conditioning the survival of aerosolised microorganisms in photocatalytic materials under actual operating conditions of air-conditioning systems. To achieve this, a reactor was specifically designed to replicate a well-characterised and -controlled experimental bioaerosol and to perform a reproducible coating of aerosolised microorganisms on different types of commercial filters. The effects of different physical parameters such as the aerosolisation time, the frontal air velocity and the relative humidity inside the reactor were assessed based on the amount of aerosolised bacterial cells retained by the filters. Finally, the survival of these cells in the photocatalytic filters was investigated immediately after their coating or after exposure to UV-A and UV-C radiation and compared to their survival in non-photocatalytic filters under the same experimental conditions.

2. Experimental

2.1. Test microorganism

The bacterium *Escherichia coli* K-12 strain MG1655 was selected for aerosol generation experiments and coating tests on filters. This rod shaped, gram-negative bacterium of dimensions 0.7–0.8 μm × 1.5 μm, was chosen as a microorganism model because it is commonly used in laboratory-based bioaerosol tests to evaluate indoor air cleaning technologies [16,17,23].

2.2. Filter materials

The two main filtering materials used for bioaerosol tests were provided by the manufacturer Ahlstrom. They are multi-layered non-woven materials, 2.5 mm thick, used as air filters in ventilation systems. They are composed of two layers of non-woven natural fibres made of cellulose and polyester fibres with an inner activated charcoal layer supplied in diameters ranging from 0.25 to 0.60 mm. The diameter of the filter fibres was about 20 μm. The immobilised photocatalyst used in this study was the commercial PC500 titanium dioxide from Cristal Global (anatase > 99%, specific surface area of 350–400 m^2/g, mean crystallite size of about 5–10 nm). Titania PC500 was coated on filters using an aqueous dispersion of colloidal silica SiO_2 as an inorganic binder (European patent). The TiO_2 coating procedure was developed by Ahlstrom [24]. For the purpose of this study, the filters without PC500 photocatalyst were named non-photocatalytic AC filters and those with a titania coating were named photocatalytic AC filters.

A third filter, 0.42 mm thick, made of a mix of fibreglass and acrylic latex with a PC500 TiO_2 coating was also used as a highly efficient particulate air (HEPA) filter.

For bacterial coating procedure with bioaerosol generation, filters were cut into discs of 10 cm of diameter and sterilised by autoclaving (120 °C for 20 min) before use.

2.3. Experimental devices

2.3.1. Reactor for the generation of the experimental bioaerosol

A specific reactor with an optimal geometry was developed to ensure the generation of aerosols of microorganisms with predefined and reproducible physical characteristics in controlled environmental conditions (relative humidity, temperature, airflow velocity) and their homogeneous coating on filters. The system included a pure air supply unit, a bioaerosol nebuliser and a reactor.

2.3.1.1. The bioaerosol nebuliser. A Collison six-jet nebuliser (BGI Inc., Waltham, MA, USA) was used to generate bioaerosols of bacterial cell suspensions with a 0.95–1.00 bar pressure. This device is commonly used to generate highly concentrated microbiological aerosols, including mainly bacteria and viruses for pulmonary models of diseases [19,23,25,26].

2.3.1.2. Pure air supply unit. An air compressor was used to supply dry and filtered high-pressure air to the Collison nebuliser inlet with an airflow equal to Dn = 8 l/min. In addition, dry and filtered

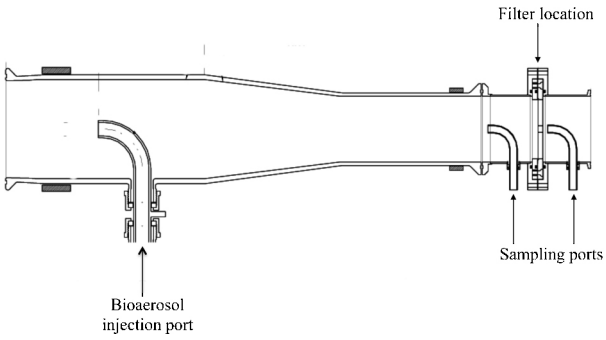


Fig. 1. Design of the straight section of the reactor.

air was injected into the glass chamber. Airflow rates were adjusted using regulated thermal mass flow metres.

2.3.1.3. The reactor. It is composed of two main parts. The first part is a glass chamber in which the Collison nebuliser provided first the bioaerosol *via* an injection port. This chamber was specifically designed to homogenise the size of the aerosolised droplets. *Via* a second injection port, the bioaerosol was then moved through a cylindrical straight section (Fig. 1) composed of a glass part and of a stainless steel unit in which the test filter was fixed. The glass part is of specific length to ensure a homogeneous coating of aerosolised microorganisms on the filter material. Toric joints between the different sections prevent aerosol leakage.

Both extremities of the straight section are surrounded by HEPA filters (H14). The upstream HEPA filter enabled a filtered air supply into the reactor while the downstream filter provided the filtration of the generated bioaerosol after going through the filter and before being released into the Microbiological Safety Cabinet. A medium pressure centrifugal fan located after the downstream HEPA filter was used to maintain a constant airflow through filters and a stable negative pressure inside the reactor, thus ensuring containment of any aerosolised microorganisms that might escape from the reactor. Two sampling ports, located upstream and downstream from the filter, were specifically designed to monitor the relative humidity, temperature and frontal velocity rates inside the reactor with specific measuring devices. Air outflow profiles were modelled using the Star CCM+ software to validate the reactor's geometry and dimensions.

The entire device was contained within a class II Microbiological Safety Cabinet (Thermo Fischer Scientific), providing a sterile atmosphere and avoiding user exposure.

2.3.2. UV radiation source

The high-pressure mercury lamp HPK 125 W with a spectral range of 200–400 nm was used as the UV radiation source. The system was cooled with a water circulation cell and equipped with two types of filters: a Corning 0.52 filter to cut off the emission spectrum below 340 nm for experiments under UV-A radiation and quartz filters to obtain radiation in the range of 200–400 nm with all UV types for experiments under UV-C radiation. The light intensity was determined through the use of a radiometer (VLX-3W, UVItec) equipped with 365 nm, 312 nm and 254 nm detectors calibrated in the spectral range of 355–375 nm, 280–320 nm and 254 nm respectively. They were set up at the same distance from the light source as from the surface of filters.

2.4. Sample preparation procedures

2.4.1. Preparation of the initial bacteria-containing suspension for bioaerosol generation and membrane filtration method

E. coli cells were inoculated in Luria Broth (LB) liquid medium and grown overnight at 37 °C with constant shaking, under aerobic condition. At a stationary growth phase, the cells were recovered after washing culture samples by successive centrifugations in sterile demineralised water. Finally, the bacterial cells were suspended in demineralised water and the bacterial suspension was diluted as required. The bioaerosol was produced with carefully washed microorganisms, as nutrients in the culture medium could have contributed to bacterial growth and salts could have modify the particle size profile of the bioaerosol. This protocol was perfected to control the reproducibility of the cultivable cell concentration. It was carried out before each bioaerosol generation test.

2.4.2. Preparation and imaging of samples for scanning electron microscopy (SEM) analysis

Samples of filters containing coated aerosolised microorganisms were fixed by glutaraldehyde in cacodylate buffer, washed with a specific buffer and dehydrated through a graded series of ethanol-water mixtures up to 100% ethanol. They were finally sputter-coated with gold and analysed by using a FEI ESEM model XL 30 scanning electron microscope or a Hitachi S800 scanning electron microscope.

2.5. Characterisation of the experimental bioaerosol

A number of flow and physical measurements and characterisation tests were performed on the reactor in order to reproduce realistic HVAC system operating conditions and generate a bioaerosol with controlled physical properties.

2.5.1. Measurement of the particle size distribution (PSD) of the bioaerosol

An Optical Particle Counter (OPC-Grimms, 1.108 model) was used for continuous real-time monitoring of the particle size distribution inside the generated bioaerosol. This instrument measured particle sizes as optical equivalent diameter (d_{opt}) in 16 different optical size ranges from 0.3 to 20 μm, with an integration counting over 6 s. Bioaerosol samples were taken through a sampling port located upstream from the filter location and isokinetic sampling conditions were achieved through the use of a sample probe of specific design. The samples were thus representative of the real particle concentration within the flow. The aerosol was drawn into the unit through an internal volume-controlled pump with a flow rate of 1.2 l/min.

2.5.2. Velocity profile test

To simulate the frontal air velocity of indoor air ventilation systems, the frontal air velocity inside the reactor was chosen to be $Vf = 0.5 \pm 0.03$ m/s. To reproduce real operating conditions, uniformity of the velocity for the filter cross-section was an important parameter to avoid airflow disruptions inside the reactor and unequal pressure drops. A series of tests was thus conducted to assess the velocity uniformity inside the reactor in accordance with the NF X 10-112 standard. Air velocity measurements were taken at each node of a six-node grid of both upstream and downstream cross-section filter by using a 435 Testo hot-wire anemometer. Triplicate tests were performed for each filter type and with different frontal velocities (minimal, medium, maximal) under monitored RH and constant temperature values.

2.5.3. Monitoring of relative humidity and temperature inside the reactor

Bioaerosol generation experiments were performed with a reactor internal temperature of 25.5 ± 0.3 °C and a relative humidity (RH) equal to 39 ± 2%, which complies with the recommended values for building and HVAC system design [27]. These parameters were monitored throughout the bioaerosol generation tests, through the downstream sampling port with a thermal anemometer. For experiments under 30% RH in the gas stream, RH was adjusted by changing the flow rate ratio of dry air.

2.6. Coating of aerosolised E. coli bacteria on filters

E. coli bacteria aerosol was continuously generated through filters for periods of 20, 40, or 60 min to achieve different amounts of aerosolised cells coatings. Immediately following aerosolisation, the test filter was removed under sterile conditions.

2.7. UV radiation exposure of filters and bacteria extraction procedure

Filters were placed under the light source, at a distance ensuring a homogeneous UV exposure of their entire surface with a total radiance of 3.6 mW/cm^2 for UV-A experiments. For inactivation experiments under UV-C radiation, the total radiance intensity was 3.6 mW/cm^2, which corresponds to the sum of UV-A, UV-B and UV-C radiance intensities. Three irradiation durations (2, 4 and 6 h) were used to assess the effects of both types of UV radiation exposure on bacterial cells coated on non-photocatalytic or photocatalytic filters. Dark control experiments with filters contaminated under the same experimental conditions but without UV light exposure were carried out at the same time. Two replicate filters were used for each time period of exposure to UV or dark conditions. *E. coli* bacteria were then extracted from the filters by a disintegration procedure in an isotonic solution.

2.8. Microbiological analysis

2.8.1. Evaluation of the number of bacterial cells

To determine the concentration of cultivable *E. coli* bacteria in aqueous suspension in the nebuliser, samples were taken and then spread onto LB agar plates after serial dilutions in demineralised water. After incubation for 24 h at 37 °C, bacterial colonies were numbered. Three replicate plates were used at each sampling time.

The quantity of microorganisms remaining on each filter, before or after UV radiation exposure, was determined, after the extraction procedure, by the dilution plating method as described above. Three replicate plates were used at each sampling time.

2.8.2. Evaluation of modifications in bacterial permeability

Damages to the bacterial outer membrane were investigated by using the LIVE/DEAD® BacLight™ bacterial viability kit according to a procedure previously described [28]. Appropriate dilutions of the stained samples were coated on 13-mm diameter, 0.4-μm pore size black polycarbonate membranes (GE Water & Process Technologies). Bacterial cells coated on air filters were labelled by the staining solution and then filters were mounted on microscope slides. Observations were performed by fluorescence microscopy using a BX51 Olympus microscope.

2.8.3. Gel electrophoresis of nucleic acids

After overnight lysis of bacterial cells in filters through a classical method previously reported, aliquots of 20 μl of the pellets were examined by electrophoresis (100 V, 20 min.) on 1% (w/v) agarose gels in TAE buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.2), stained with ethidium bromide (3 μl/100 ml) [29]. After migration of samples, the gels were observed under UV illumination and photographed.

2.9. Data interpretation

The bioaerosols generated were described by a measure called count median diameter (CMD), which is the particle diameter that halves the count particle size distribution. The distribution around the CMD is expressed in terms of geometric standard deviation (GSD). It provides a representation of the uniformity of the particles and was obtained by dividing the CMD by the particle size of 84.13% probability [30]. Aerosols with GSD above 1.2 are considered polydisperse.

3. Results and discussion

3.1. Characterisation of the bioaerosol

3.1.1. Measurement of the particle size distribution

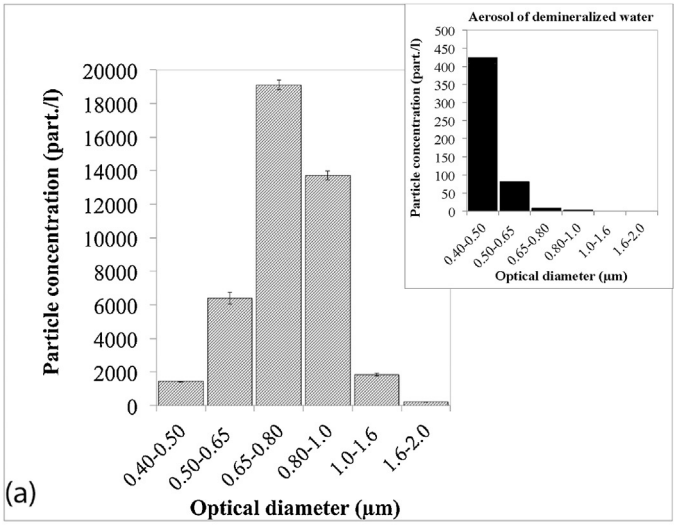
The first step in our study was to investigate the particle size distribution (PSD) of the bioaerosol, which provides relevant information about the dispersion of the bacterial cells in the aerosolised particles. It was compared with the PSD of an aerosol of demineralised water in order to determine the proportion of particles arising from potential water impurities. Both profiles of Fig. 2(a) correspond to the distributions generated upstream of a photocatalytic AC filter over 60 min of bioaerosol generation. Identical profiles were obtained with the non-photocatalytic AC filter.

We observed that the presence of demineralised water did not influence the PSD of the bioaerosol (insert Fig. 2(a)). Indeed, at 39 ± 2% RH, the optical diameter of water particles was mainly included within the range of 0.4–0.5 μm. In contrast, the measured PSD of the bioaerosol indicated the presence of two major populations of particles: one within the optical diameter size range of 0.65–0.80 μm and another ranging from 0.80 to 1.00 μm. The counted median optical diameter of the aerosolised particles within the bioaerosol was 0.75 μm. As a result, the particle populations with an optical diameter located in the range of 0.65–0.80 μm mainly contained the aerosolised *E. coli* bacteria. As the geometric standard deviation was equal to 1.23 across all the experiments performed, the bioaerosol was considered polydisperse. This could be explained either by the rod-shaped morphology of *E. coli* cells or by their heterogeneity within the initial bacterial culture. In fact, heterogeneity in terms of length and diameter is one of the main properties of natural bacterial populations as shown in Fig. 2(b). The presence of the particle population with optical diameters of 0.50–0.65 μm could be justified by the orientation of the bacterial cells when they go through the laser-measuring cell of the OPC. The smaller particles present in this aerosol within the range of 0.40–0.50 μm could be composed of residual impurities of generated water particles (approximately 30%) and fragments arising from bacterial cells damaged by the aerosolisation process. The particle population with a larger optical diameter (from 1.00 to 2.00) was probably due to the tendency of bacterial cells to aggregate.

Information on the evolution of the physical parameters of bioaerosols over time is either unavailable or incomplete in most studies addressing the inactivation of aerosolised microorganisms by photocatalysis. Our results demonstrate the ability of the nebuliser to generate an aerosol of *E. coli* cells with a distribution of particle sizes clearly physically realistic.

3.1.2. Effect of the physiological state of the aerosolised cells on the PSD

To go further in our investigation, we studied the evolution of the concentration of the particle populations that mainly contained



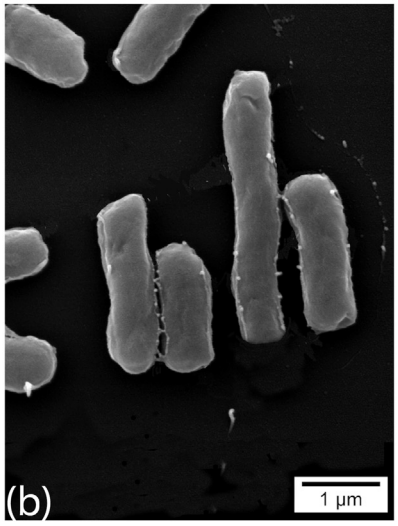


Fig. 2. (a) Particle size distribution for the bacterial aerosol measured with an OPC-Grimms within isokinetic conditions. Insert: Particle size distribution for an aerosol of demineralised water. (b) SEM picture of stationary-phase *E. coli* cells in LB medium.

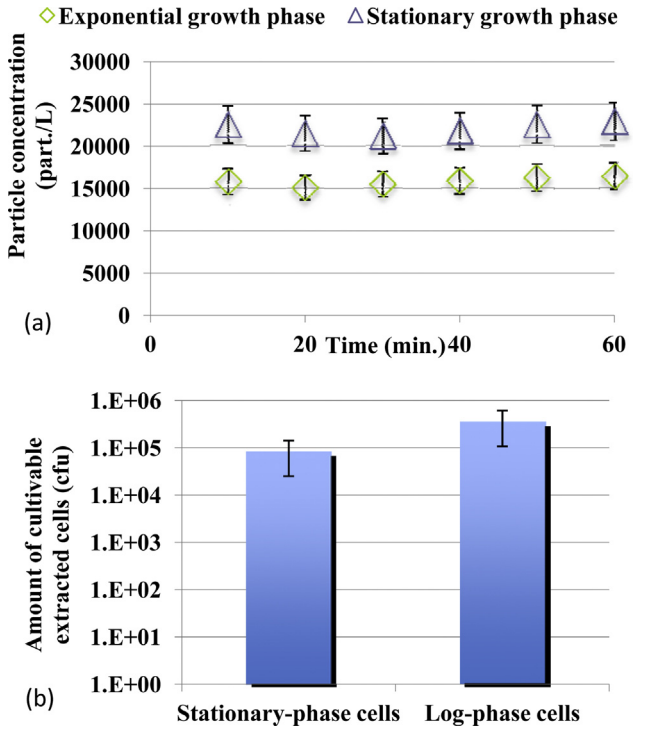


Fig. 3. (a) Evolution of the concentration of the particle population with an optical diameter of 0.65–0.80 µm from bioaerosols generated from suspensions of: exponential growth phase *E. coli* bacteria (◊); stationary growth phase *E. coli* bacteria (▲). (b) Amount of cultivable bacterial cells extracted from filters contaminated with aerosolised log-phase cells or stationary-phase cells.

aerosolised bacterial cells throughout a 60-min period of aerosolisation (Fig. 3(a)). Two different bioaerosols were thus generated, one from an initial suspension of *E. coli* bacteria removed from an exponentially growing (log-phase) culture and another from a bacterial suspension with cells that had reached a stationary growing phase. These experiments were performed in triplicate to check the reproducibility. Although both suspensions had the same initial concentration (1.5×10^8 cfu/ml), the particle concentration from the aerosol of bacterial cells collected from stationary phase culture was significantly lower than the particle concentration detected in the aerosol of bacterial cells taken in exponential growth phase.

Moreover, a higher number of cultivable bacterial cells was extracted from filters contaminated with aerosols generated with stationary-phase cells than from those contaminated with the log-phase cells (Fig. 3(b)). The physiological state of microorganisms has consequences on their ability to survive through many adverse environmental conditions. Bacterial cells in log-phase of growth are generally more sensitive to stresses than stationary-phase cells [31,32]. Yet, to our knowledge, so far little attention has been given to the physiological difference between grown and starving cells as a possible factor affecting microorganism viability during aerosolisation [33]. Our results suggested that log-phase bacterial cells appeared to be more susceptible to stress introduced by continuous aerosolisation. Bacterial cells could exhibit different levels of survival response to aerosolisation with regards to their physiological state which might play an important role in aerosol survival, persistence, and subsequently, resistance when they go through the filters. Since log-*E. coli* cells were expected to be more sensitive to the aerosolisation stresses than the stationary ones, these bacterial cells were not used for further experiments.

3.1.3. *Assessment of the stability of the particle size distribution over time*

The minimal duration required to obtain stable concentrations of particle populations during aerosolisation was determined. This is crucial information to ensure the stability of the particle concentrations, in particular for those containing bacterial cells, prior to the coating of aerosolised *E. coli* cells on filters. Fig. 4(a) represents the evolution of the particle concentrations over the first 10 min of bioaerosol generation inside the reactor. We observed a stabilisation of the concentration for all particle populations after 5 min of aerosolisation.

We then focused on the ability of the bioaerosol nebuliser to maintain stable particle size distribution overtime by studying the evolution of the aerosolised particle concentration over 60 min of aerosol generation. Results of Fig. 4(b) showed that the particle concentrations remained stable for the first 60-min period. These results were made reproducible between tests. We can thus conclude that the generation of a stable bioaerosol was made possible for 60 min with defined, repeatable and reproducible physical characteristics in controlled atmosphere (relative humidity, temperature, airflow velocity).

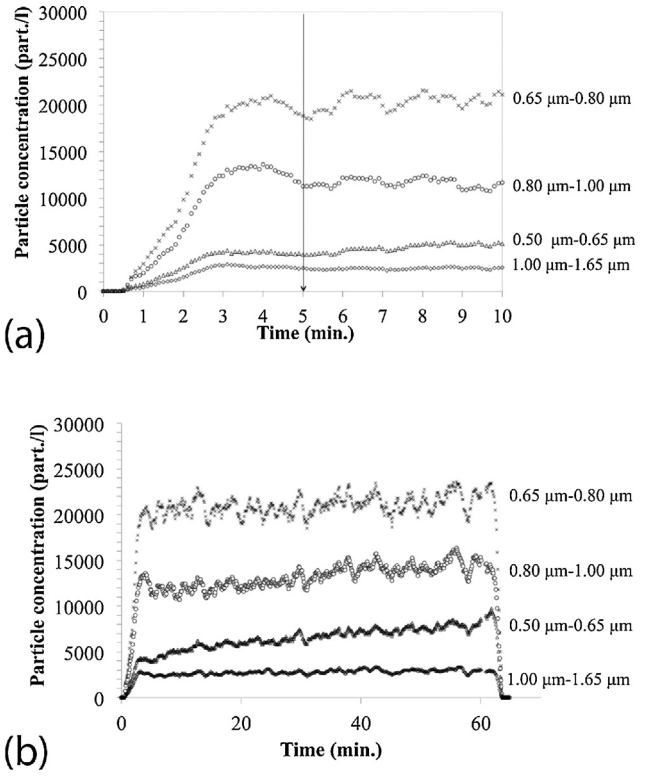


Fig. 4. Evolution of the concentration of the aerosolised particle populations from the bioaerosol over: (a) the first 10 min of generation and (b) a 60-min period of generation.

3.2. Microbiological characterisation of the bacterial suspension in the nebuliser

A number of articles have reported that the generation of aerosol through a Collison nebuliser could have deleterious effects on bacterial cells mainly through desiccation, impaction and shear forces [34,35]. Moreover, initial washings and suspension of *E. coli* bacteria in demineralised sterile water rather than in a saline solution could also damage the bacterial physiological state and especially weaken the outer membrane permeability, even before the start of the aerosolisation procedure in the reactor.

The cultivability on a standard growth medium of *E. coli* cells in suspension inside the Collison nebuliser, as well as their outer-membrane integrity, were thus assessed before or after 30 and 60 min of aerosolisation (Fig. 5).

In the original aqueous suspension, only about $5 \pm 2.3\%$ of *E. coli* cells were found with injured outer membrane by using LIVE/DEAD® BacLight™ bacterial viability kit. Suspension in demineralised water did not have significant deleterious effect on the bacterial permeability. Over the first 30 min of generation, the percentage of membrane-damaged cells remained stable (from 5 to $5.4 \pm 2\%$). Thereafter, a limited increase of approximately $15 \pm 4.5\%$ was noticed after 60 min of aerosolisation. However, no cultivability loss was observed during this period, which suggests that microorganisms are not damaged enough to lose their ability to grow on their nutrient medium. Liquid disruption was not as deleterious as reported in the literature [34,35]. Therefore, in our experimental conditions, the Collison nebuliser induced limited metabolic injuries to *E. coli* cells over prolonged generation and allowed the generation of bioaerosol from microorganisms with proper physiological state.

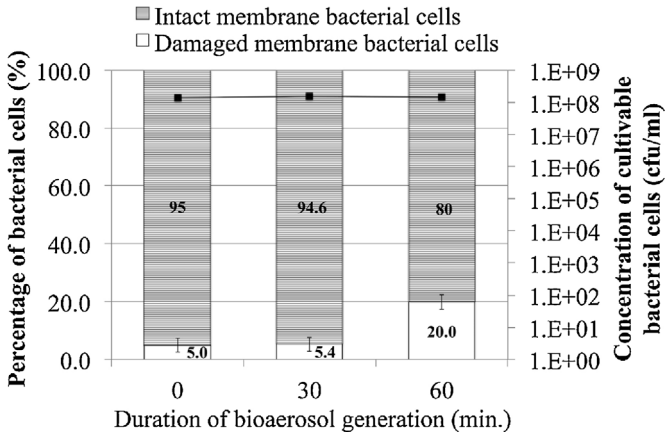


Fig. 5. Evolution of the cultivability on LB medium ((■) symbol curve) and of the permeability (bar chart) of *E. coli* bacteria in aqueous suspension inside the nebuliser before (0) or after two periods of bioaerosol generation with Dn = 8 l/min, $P = 1$ bar.

3.3. Coating of aerosolised bacterial cells on filters

3.3.1. Assessment of the effect of the extraction procedure on microorganism viability

The potential mechanical stress of the extraction step on microorganism viability was first analysed by applying the procedure to bacterial suspensions of different concentrations. No change in cultivability and membrane permeability of the microorganisms was observed before and after the disintegration procedure, thus demonstrating no damages arising from the extraction step.

The efficiency of this procedure at removing microorganisms from filters was also assessed by coating in triplicate the same *E. coli*-containing suspensions on the three types of filters using the membrane filtration technique, thus eliminating any stress due to the aerosolisation process. After the extraction procedure by disintegration, the ratio of cultivable bacterial cells was $95 \pm 2.3\%$ for the non-photocatalytic AC filters and $95 \pm 3.7\%$ for the photocatalytic AC filters. Due to a thinner layer, the extraction ratio was of $97 \pm 1.8\%$ for the photocatalytic HEPA filters. These results revealed a good efficiency to remove almost all of the coated microorganisms. This is an important point to rigorously assess the disinfection efficiency of different types of photocatalytic materials.

3.3.2. Study of the coating of aerosolised bacterial cells on filters - Effect on their membrane integrity

As shown on Fig. 6, the amount of aerosolised bacterial cells extracted from both AC filters after three periods of aerosolisation increased as duration increased and appeared to be reproducible between tests within the same experimental aerosolisation conditions. A higher penetration of *E. coli* bacterial cells was observed for the photocatalytic filter regardless of the aerosolisation duration. The efficiency of a filter usually depends on its thickness, its compacity and the diameter of its fibre. These three parameters remain the same for both types filters. Therefore, the titanium dioxide deposit, which induces a more significant loss of charge and tighter pores for the photocatalytic filter, could explain its better filtration efficiency.

Considering the thickness of the filters (2.5 mm for both types), the concentration of extracted microorganisms should have been expressed based on the total volume of the filters. However, concentrations of bacterial cells extracted from non-photocatalytic or photocatalytic filters were calculated by the standard method namely per square centimetre of filter material (Table 1) in order to be comparable with results reported in the literature.

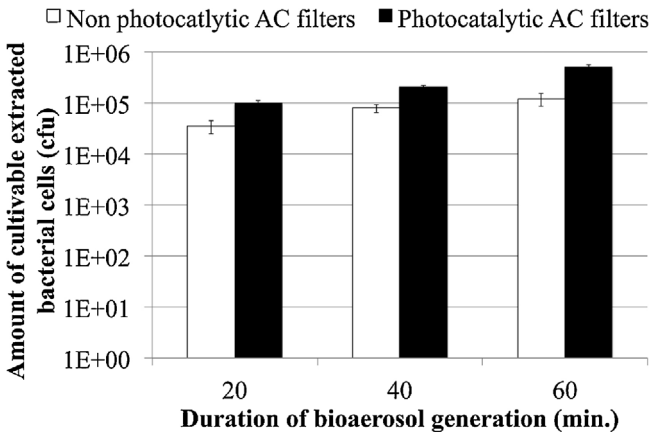


Fig. 6. Amounts of cultivable *E. coli* bacteria extracted for both types of filters immediately after 20, 40 or 60 min of bioaerosol generation.

The values obtained in Table 1 are slightly higher than those previously reported in studies addressing the filtration of air in real environment (clean rooms, industry, buildings). As mentioned above, this could be justified by the better filtration efficiency of the photocatalytic filter due the titanium deposit. This result could also be explained by the efficiency of our extraction procedure at removing higher numbers of aerosolised bacterial cells with lesser deleterious impact on their physiological state, thereby increasing the amount of cultivable extracted bacteria. Moreover, our results are representative of the total quantity of microorganisms that filters could accumulate throughout their expected lifetime.

However, taking into account both the airflow inside the reactor and the frontal velocity, the total number of aerosolised bacterial cells coated on a filter after 60 min of bioaerosol generation should be equal to approximately 3×10^8. The analysis by gel electrophoresis of the nucleic acids of aerosolised cells extracted from a photocatalytic AC filter after 60 min of bioaerosol generation confirmed this number (Fig. 7(a)). Indeed, the picture of the gel electrophoresis of the nucleic acid showed the presence of three distinct bands corresponding to the presence of bacterial chromosomic DNA and 23S and 16S rRNA. The total amount of double-stranded DNA extracted from this filter was evaluated through a spectrophotometric method to be equal to 3.6 μg. Since no other band was observed on the gel, this DNA quantity can only be attributed to the presence of *E. coli* bacteria. Moreover, considering that the percentage of DNA by dry weight of *E. coli* cell was 3%, and that the dry weight corresponding to one *E. coli* cell was 1.2×10^{-12} g, the amount of cells extracted from the photocatalytic filter after 60 min of aerosolisation was estimated to be 1×10^8 cells [36]. This value is very similar to the theoretical amount calculated above but higher than the number of cultivable bacterial cells numerated within the same experimental conditions (Fig. 6). This difference could be explained by the presence of injured *E. coli* cells that probably lost their ability to grow on culture medium due to the stress of the aerosolisation.

Table 1
Concentration of aerosolized *E. coli* bacteria per square centimetre of filters after 20, 40 and 60 min of bioaerosol generation.

Aerosolisation duration (min)	Concentration of extracted aerosolised bacteria (cm^2)	
	Non-photocatalytic filters	Photocatalytic filters
20	0.45×10^3	1.3×10^3
40	1.0×10^3	2.6×10^3
60	1.5×10^3	6.4×10^3

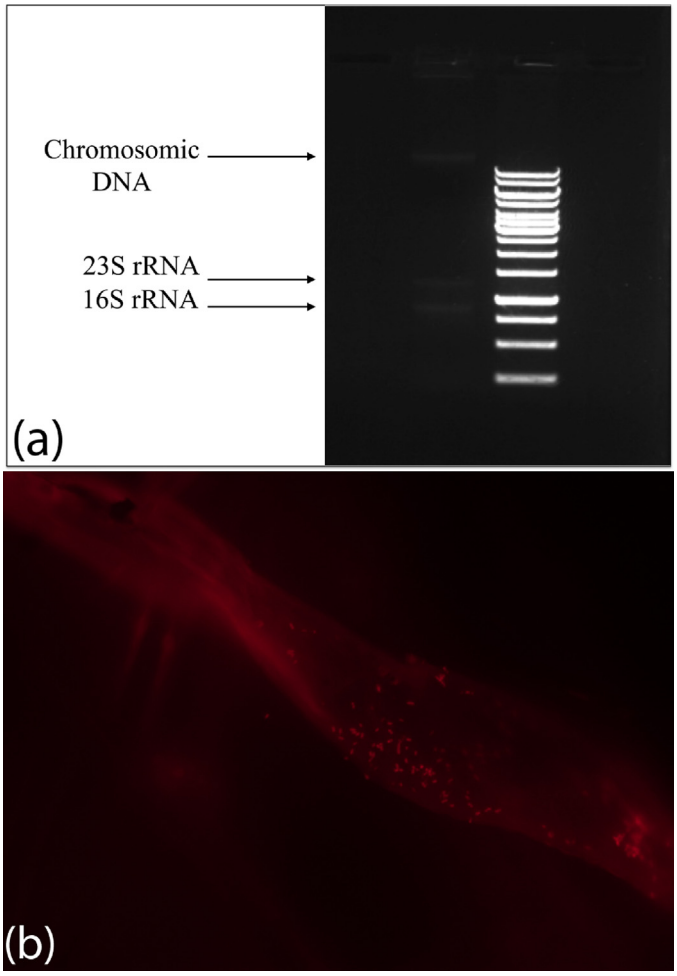


Fig. 7. (a) Gel electrophoresis of nucleic acids of aerosolised *E. coli* bacteria extracted from a photocatalytic AC filter after 60 min of bioaerosol generation. (b) Epifluorescent microscopy picture of aerosolised *E. coli* cells stained according to a modified BacLight™ kit procedure inside a photocatalytic AC filter after 20 min of bioaerosol generation.

To go further in our investigation, we also study the membrane integrity of aerosolised *E. coli* bacteria retained by filters during the aerosolisation process. Thus, we developed a procedure to stain bacterial cells directly inside filters using the LIVE/DEAD® BacLight™ bacterial viability kit. The fluorescent microscopy picture of Fig. 7(b) shows *E. coli* cells stained immediately after a 20-min period of aerosolisation and located on the fibres of a photocatalytic AC filter. The picture in Fig. 7(b) was representative of the entire surface of the samples observed. The permeability of the aerosolised bacterial cells was damaged and the same observation was made for both filter faces as well as for their inner layer. The same results were obtained with the non-photocatalytic AC filter and for 40 and 60 min of bioaerosol generation. Under our experimental conditions, we can thus conclude that aerosolisation and penetration through filter induced significant stresses that affected the bacterial outer membrane even if *E. coli* cells remained cultivable. This result could explain the difference between the number of cultivable cells and the number of total bacterial cells extracted from filter. As a result, the bacterial permeability was not assessed further under UV radiation exposure.

3.3.3. *Effect of relative humidity and frontal velocity in the reactor on the number of aerosolised bacteria retained by filters*

We investigated the effect of two different RH (30% and 40%) on the amount of microorganisms retained by the photocatalytic AC

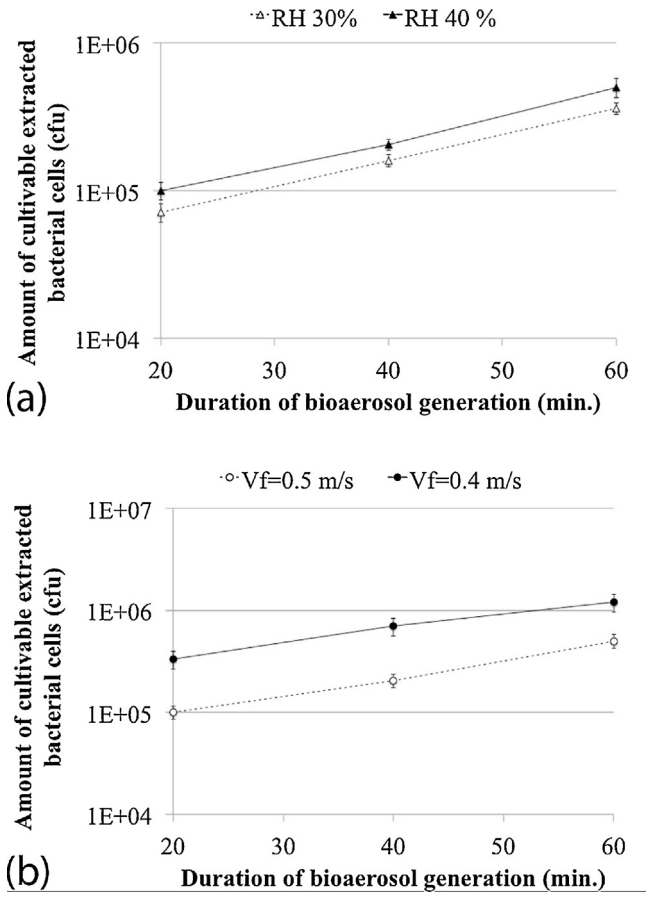


Fig. 8. Amounts of cultivable *E. coli* bacteria extracted for photocatalytic AC filters after 20, 40 or 60 min of bioaerosol generation according to (a) RH level of 40 ± 2% or 30 ± 2%; (b) frontal velocity with Vf = 0.5 ± 0.03 m/s or Vf = 0.4 ± 0.03 m/s.

filters (Fig. 8(a)). These values correspond to those usually encountered in indoor environments with ventilation systems where acceptable RH levels should range between 30% and 70%. The impacts of two levels of air velocity of 0.4 and 0.5 ± 0.03 m/s were also assessed (Fig. 8(b)).

An increase of RH from 30% to 40% induced a limited increase in the amount of bacterial cells retained by filters. No subsequent change in the counted median optical diameter within this RH range was observed. One factor that can adversely affect survival is desiccation, which usually occurs during aerosolisation of microorganisms with low-level RH. Thus, our results were probably due to the protective action of humidity against desiccation of the cells during aerosolisation at 40% RH. In contrast, the retention of microorganisms by the photocatalytic filters increased as frontal velocities decreased, whatever the period of generation of the bioaerosol. This can be explained by a lesser deleterious impact for the bacterial cells when they go through the filters. As a result, the same number of aerosolised cells would be coated on filters for both velocity values but a higher number of cells would remain cultivable for a lower velocity. We could also formulate the hypothesis that a lower filter face velocity does not break aggregates of cells, which might then be subject to a higher filtration efficiency of the filter material.

3.3.4. *Survival of aerosolised bacterial cells in filters in the dark or under UV radiation exposure*

A series of experiments was then carried out to analyse the survival of aerosolised *E. coli*-cells in each type of filter material, in the dark or under UV-A or UV-C radiation exposure (graphs of Fig. 9(a)

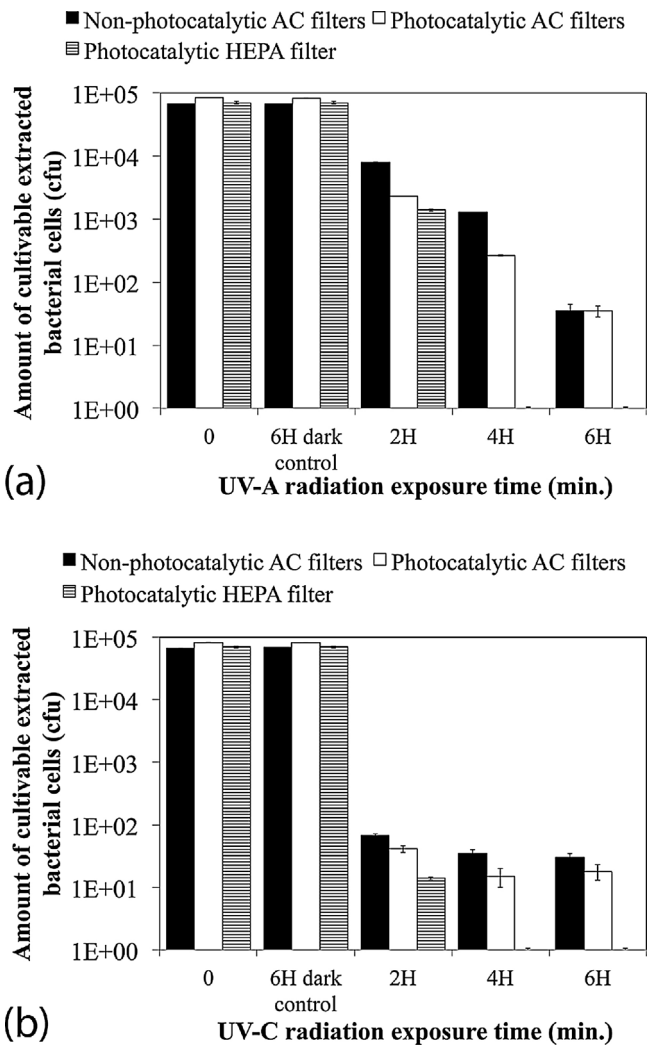


Fig. 9. Amount of cultivable bacterial cells extracted from non-photocatalytic or photocatalytic AC filters after 60 min of bioaerosol generation and 2, 4 or 6 h of exposure of filters to UV-A (a) or UV-C (b) radiation.

and (b)). At the same time, SEM was used to check the presence of aerosolised bacterial cells and identify their morphological changes in photocatalytic AC filters after aerosolisation or after 6 h of exposure to UV-A or UV-C radiation (Fig. 10). The pictures in Fig. 10 were representative of the samples observed on the microscopic sampler holder.

After aerosolisation, the general rod-shaped morphology of *E. coli* cells appeared to be normal (Fig. 10 picture (a)). In some cases, their outer-membranes were damaged probably due to the stress of aerosolisation and the contact with fibres, thus explaining the loss of cultivability of some microorganisms immediately after their coating on filters (Fig. 10 picture (b)). After 6 h of contact with both filters in the dark, all bacterial cells initially numerated remained cultivable. However, after 24 h in the dark, all cells lost their cultivability but were still visible on microscopic pictures for more than 30 days. Moreover, when contaminated materials incubated in the dark for 30 days were put in suspension in a rich liquid medium in optimal incubation conditions, bacterial growth was clearly observed after 24 h, thus indicating that the cells maintained a metabolic activity and survived inside filters without any source of nutrients.

Under UV-A radiation, the photocatalytic HEPA filter was the most efficient at fully inactivating *E. coli* bacteria after 4 h of UV-A exposure. As regards filters with activated carbon, a faster

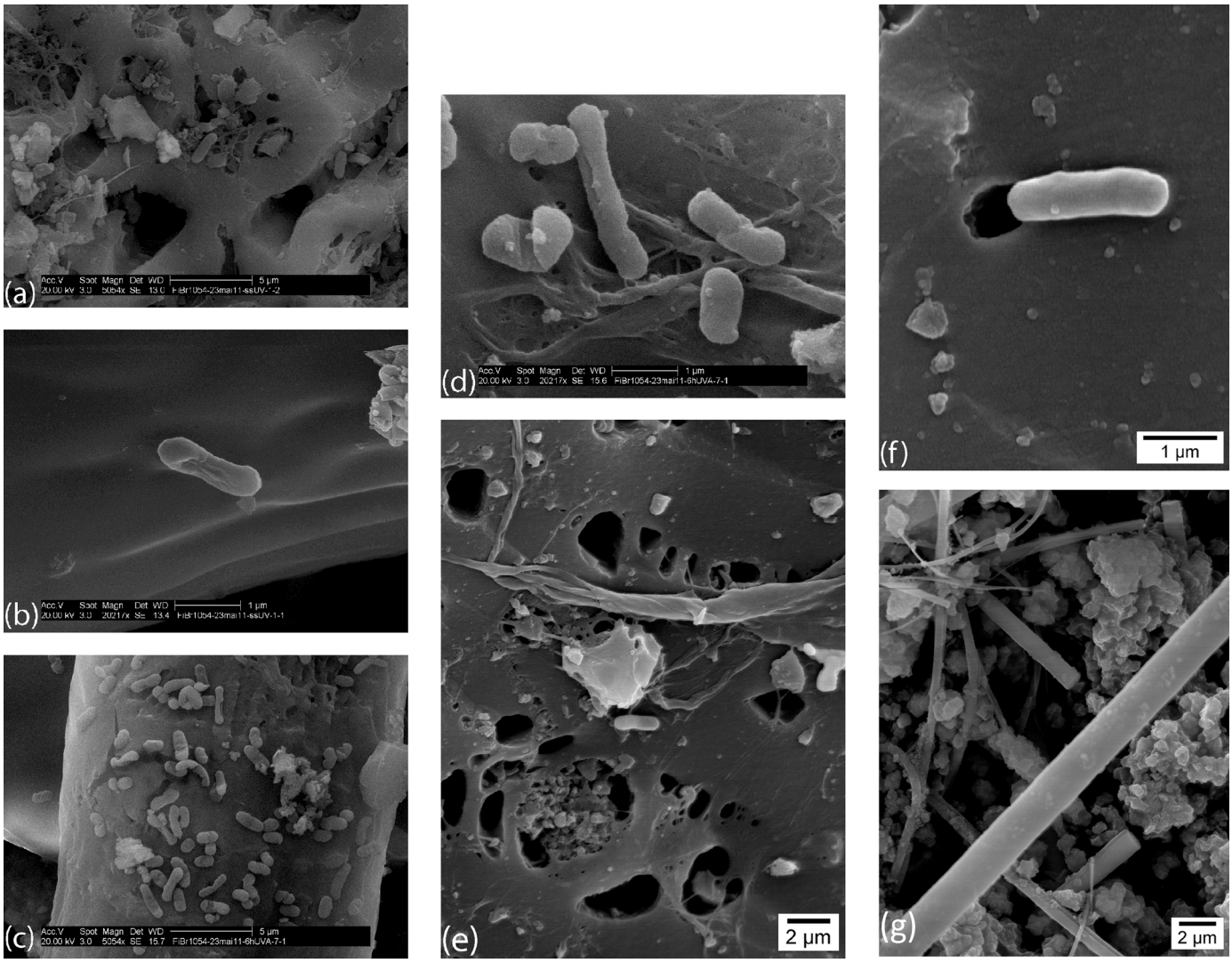


Fig. 10. SEM pictures of aerosolised *E. coli* cells in: photocatalytic AC filters immediately after aerosolisation (a) and (b), after 6 h of exposure to UV-A radiation (c) and (d), after 6 h of exposure to UV-C radiation (e) and (f); SEM pictures of photocatalytic HEPA filters after 6 h of UV-C radiation (g).

inactivation of cultivable bacterial cells was observed for the photocatalytic material for short exposures (2 and 4 h) (Fig. 9(a)). Among all UV types, UV-A radiation is known to be the least efficient to inactivate microorganisms as it is not directly absorbed by nucleic acids [37]. However, in the literature it is demonstrated that oxidation reactions that mainly damage DNA molecules (mostly bases modifications and DNA strand breaks) can occur under UV-A radiations [38]. Knowing that nucleobases weakly absorb wavelengths above 320 nm, most of the damaging effects of UV-A on cellular DNA involves photosensitisation reactions leading to photo-oxidation within cells [39].

The loss of cultivability of bacterial cells under UV-A radiation in the non-photocatalytic AC material could thus be explained by both the aerosolisation stress, the contact with fibres but also the action of UV-A. After 6 h of UV-A radiation, similar amounts of bacterial cells extracted from each filter were still cultivable. The similar amount of bacteria found after 6 h of UV-A in presence of photocatalytic and non-photocatalytic AC filter could be explained considering that bacteria are probably retained inside the inner activated charcoal layer of AC filters and not exposed to UV-A.

On Fig. 10(c) numerous cells were visible but the majority had their overall structure fairly damaged as clearly observed on Fig. 10(d). Some microorganisms were entirely deformed with parts of the bacterial envelope missing (Fig. 10(d)) while others exposed a typical morphology. Moreover, it is important to note that cells numerated on microscopic pictures were in greater number than the ones numerated on growth medium. This suggested the presence of non-cultivable cells inside filters that classical numeration methods were not able to detect.

When filters were exposed to UV-C radiation, we noticed an evident faster inactivation of the cells in comparison with UV-A radiation, probably due to the germicidal action of UV-C radiation. The photocatalytic HEPA material proved again to be more efficient at inactivating the aerosolized microorganisms after 4 h of UV-C and no cell was visible on SEM figures (g). The efficiencies of the two filters with an activated charcoal layer were both similar even if the bacterial loss of cultivability was slightly faster for the photocatalytic material (about 0.5 log). However, the amount of cultivable cells did not evolve significantly between the first and the last occurrence of UV exposure, thus indicating an inactivation threshold under UV lights, not observed in the case of the HEPA filter. We could thus make the hypothesis that the bacterial cells still cultivable after 6 h of UV radiation were probably those retained inside the inner activated charcoal layer of AC filters. Activated carbon is a common adsorbent used in filter media to remove toxic chemicals from air. However, it only retains pollutants, but it

is not able to kill certain types such as biological pollutants. As a result, cells located in the AC layer were protected from the action of UV radiation or not exposed to a sufficient UV dose to be permanently damaged. Moreover, reactive species photogenerated at the surface of TiO_2 cannot damage the microorganisms as they were not in direct contact with the photocatalyst at the surface of the filters [40]. Although similar amounts of cultivable bacterial cells were numerated in photocatalytic AC materials after 6 h of UV-A or UV-C radiation, only a few bacterial cells were still visible on microscopic figures in the case of UV-C exposure (figures (e) and (f)). This behaviour is explained considering that bacterial cells present inside the filter are not irradiated and that the number of bacterial cells inside the filter does not depend on the presence of TiO_2. Moreover, the less important number of bacterial cells observed by microscopy under UV-C is probably due to a possible mineralisation of bacteria in contrast with UV-A, which would only induce a loss of cultivability.

4. Conclusion

The design of a reactor of specific geometry ensured the generation of reproducible aerosols of *E. coli* bacteria and their coating on photocatalytic or non-photocatalytic filters, within realistic operating environment of HVAC equipment. The characterisation of the bioaerosol in terms of physical parameters highlighted the ability of the whole device to generate stable and reproducible aerosols of bacterial cells during extended periods of time under parameters usually encountered in indoor environments.

The aerosolisation stress and the contact with fibres when the cells went through filters generated deleterious effects on the bacterial outer membrane even for short aerosolisation durations. The study of survival of the bioaerosols inside filter materials in the dark revealed the presence of resilient microorganisms, able to maintain metabolic activities even after a 30-days period without any source of nutrients. These results demonstrated the outstanding ability of bacteria cells to adapt and resist to the aerosolisation stress and to remain viable inside filters over extended periods of time. In the presence of dust, which can act as nutrients, and within sufficient humidity levels, these microorganisms could potentially colonise filters, which could in turn become a source of biological pollutants inside ventilation equipment. In view of this, culture-based methods may significantly underestimate exposure to the total population of viable aerosolised microorganisms. Under UV-A and UV-C radiation, the photocatalytic HEPA filters demonstrated a better efficiency with a total inactivation of the microorganisms after 4 h of UV exposure. The presence of an inner activated charcoal layer in other filter materials allowed the survival of aerosolised bacterial in spite several hours of exposure to UV-A or UV-C radiation. Our results highlighted the difficulties to combine the adsorption properties of activated charcoal and the microbicidal action of photocatalysis. However, activated carbon could turn out to be a crucial component to avoid the release of toxic organic compounds resulting from the photocatalytic inactivation of the aerosolised microorganisms.

Our study demonstrated that the survival of bioaerosols inside photocatalytic filters remains a crucial point to be studied. However, given that the microorganism concentrations used in this study are higher than the usual concentrations of microorganisms in indoor air, our results remain very promising for the development of photocatalysis as an air treatment technology. For further research, the development of new types of filter materials with high filtration efficiency to improve the contact between microorganisms, TiO_2 coating and UV exposure should be studied. Moreover, a lot of information critical to the understanding and conception of efficient and reliable air purification systems, still needs to be collected in realistic conditions with regards to degradation mechanisms and toxic intermediaries potentially created during the process.

Acknowledgments

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